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A1

(54) Title: PROCESS FOR THE PREPARATION OF A DIPEPTIDE AND INTERMEDIATE PRODUCT IN SUCH A PROCESS

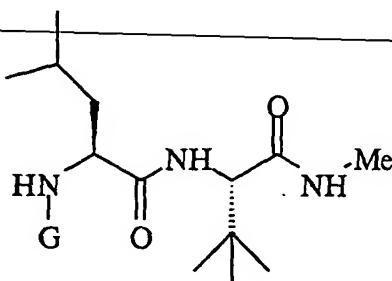
(57) Abstract: Process for the preparation of an N-formyl-L-leucyl-L-*tert*-leucine-N-methylamide in which N-formyl L-leucine is coupled to L-*tert*-leucine-N-methylamide in the presence of an activating agent. Preferably, use is made of L-*tert*-leucine-N-methylamide with an enantiomeric excess greater than 98 % and N-formyl-L-leucine with an enantiomeric excess greater than 98 %. If desired, the dipeptide obtained is subsequently deformylated and the resulting N-formyl-L-*tert*-leucine-N-methylamide or the L-leucyl-L-*tert*-leucine-N-methylamide is further subjected to one or more crystallizations. The invention also relates to the use of N-formyl-L-leucyl-L-*tert*-leucine-N-methylamide in the preparation of a dipeptide and the use of N-formyl-L-leucyl-L-*tert*-leucine-N-methylamide in the preparation of an intermediate product.

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5 PROCESS FOR THE PREPARATION OF A DIPEPTIDE AND  
INTERMEDIATE PRODUCT IN SUCH A PROCESS

10 The invention relates to a process for the preparation of a dipeptide of formula 1



(1)

15 in which G represents a protective group, with N-protected L-leucine being coupled to L-tert.-leucine-N-methylamide in the presence of an activating agent.

WO-A-96/11209 discloses such a process in which N-(1,1-dimethylethoxy)carbonyl-L-leucine and L-tert.-leucine-N-methylamide are coupled.

20 A drawback of the known process is that it uses an expensive protective group, so that the process is less attractive from a commercial point of view. The present invention provides a commercially attractive route for the preparation of the above-mentioned intermediate product in the preparation of, for instance, the pharmaceuticals as described in WO-A-25 96/11209.

This is achieved according to the invention by using a formyl group as protective group.

30 Dipeptide couplings involving the coupling of two amino acids are generally known and are described

in detail in the literature. In these couplings the activated acid group of the eventual N-terminal amino acid reacts with the amino group of the eventual C-terminal amino acid or amino acid derivative. In this 5 process the amino group of the eventual N-terminal amino acid is protected by means of a protective group.

In the process according to the invention two enantiomer-enriched amino acids are coupled. The ~~enantiomeric excess of the enantiomer-enriched amino~~ 10 acids is preferably greater than 80%, in particular greater than 90%, more in particular greater than 98%. It is known that racemization of the N-terminal amino acid may take place when the amino acids are coupled. This is the case in particular when a formyl protective 15 group is used, such as for instance described in the handbooks Houben-Weyl, Band 15/1 (1974), p. 166, and The Peptides, Academic Press 1979, Volume 1, p. 279. As a consequence, formyl protective groups are not considered for coupling of enantiomer-enriched amino 20 acids. Applicant has now found that no racemization or only a low degree of racemization takes place when the coupling is carried out according to the invention, with a formyl group being used as protective group. Moreover, applicant has found that, should racemization 25 take place, this very coupling product according to the invention is particularly suitable for enrichment in the desired diastereomeric form through crystallization.

An added advantage of the process according 30 to the invention is that inexpensive activating agents can be used in the process.

The N-formyl-L-leucine that is used in the

process according to the invention can for instance be prepared in a known manner by contacting L-leucine with formic acid and for instance an anhydride. Preferably, use is made of acetic anhydride.

5           The L-tert.-leucine-N-methylamide can for instance be prepared from L-tert.-leucine via the conversion of L-tert.-leucine and phosgene into L-tert.-leucine-N-carboxyanhydride, which is subsequently converted into L-tert.-leucine-N-methylamide with the  
10           aid of N-methylamine.

In the process according to the invention the N-formyl-L-leucine is activated by means of an activating agent, preferably a sterically hindered acid chloride or an alkyl chloroformate, and a base. Such  
15           activation steps are generally known and are often applied in peptide couplings. The bases to be used therefore are preferably the known bases used in these activation steps, with a low degree of racemization occurring. Preferably, N-methylmorpholine is used as  
20           base.

The temperature at which the activation is carried out is not very critical and in practice usually lies between -30°C and +30°C, preferably between -20°C and +10°C.

25           If desired the activation is carried out in a solvent, preferably one that is inert in the reaction mixture. Examples of solvents esters are esters, in particular ethyl acetate, isopropyl acetate and isobutyl acetate, ethers, in particular tetrahydrofuran  
30           (THF), methyl-tert.-butylether (MTBE) and dioxane, and nitriles, in particular acetonitrile.

In one embodiment first the activation is

carried out followed by a coupling step. For the coupling, the activated N-formyl-L-leucine is contacted with the L-tert.-leucine-N-methylamide. Preferably, a solution of L-tert.-leucine-N-methylamide is used.

5 In principle, for the temperature at which the coupling takes place the same holds as for the temperature at which the activation is carried out. Preferably, the coupling temperature is about the same as the activation temperature. Examples of suitable

10 solvents for the L-tert.-leucine-N-methylamide are alcohols, in particular methanol, ethanol and isopropanol, esters, in particular ethyl acetate, isopropyl acetate and isobutyl acetate and ethers, in particular THF, MTBE and dioxane.

15 Alternatively a one stage procedure may be followed for the activation and the coupling, wherein the N-formyl-L-leucine, the L-tert.-leucine-N-methyl amide and the base are solved in a suitable solvent as described above, and the activating agent is added to

20 the solution.

The resulting N-formyl-L-leucyl-L-tert.-leucine-N-methylamide can subsequently be deformylated in a generally known manner, for instance in an acid environment. The deformylation can for instance be

25 carried out in an aqueous environment, in water/alcohol mixtures or in a two-phase system.

The temperature at which the deformylation is carried out for instance lies between 20°C and 110°C, preferably between 40°C and 80°C.

30 The resulting N-formyl-L-leucyl-L-tert.-leucine-N-methylamide or L-leucyl-L-tert.-leucine-N-methylamide can if desired be purified, for instance by

subjecting it to a crystallization. Surprisingly, it has been found that the enantiomeric excess of the N-terminal amino acid in the protected or non-protected dipeptide can be increased by the crystallization in 5 those cases in which racemization has taken place during the peptide coupling.

Examples of suitable solvents that can be used in the crystallization are hydrocarbons, in particular heptane and hexane; esters, in particular 10 isopropyl acetate, isobutyl acetate and ethyl acetate; ethers, in particular MTBE; alcohols, in particular methanol, ethanol, isopropanol and butanol; or mixtures thereof. An example of a suitable mixture of solvents is a mixture of heptane and isopropyl acetate.

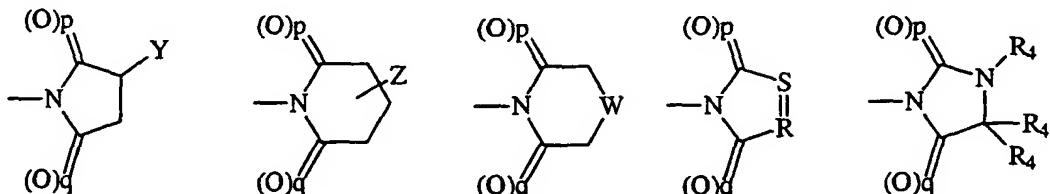
15 The temperature at which the crystallization is carried out is not particularly critical and depends mainly on the physical parameters of the chosen solvent, particularly the boiling point. In practice, the crystallization will usually be 20 carried out at a temperature between 20°C and 100°C.

Depending on the exact embodiment of the peptide coupling, it may be advantageous to isolate the N-formyl-L-leucyl-L-tert.-leucine-N-methylamide intermediate product obtained, for instance via 25 extraction or crystallization.

The L-leucyl-L-tert.-leucine-N-methylamide obtained can for instance be applied in the preparation of pharmaceuticals, for instance the N-( $\alpha$ -optionally substituted mercaptocarboxyl)- L-leucyl-L-tert.-leucine-N-methylamide compounds such as described in 30 WO-A-96/11209 and WO-A-97/12902. The  $\alpha$ -optionally substituted mercaptocarboxyl group for instance

represents a group of formula  $R_1S-C(R_2)-C(O)-$  where  $R_1$  stands for H or  $R_3CO$  where  $R_3$  is a  $C_{1-4}$  alkyl,  $(C_{1-4}$  alkyl)aryl group,  $(C_{1-6}$  alkyl)heteroaryl group,  $C_{3-6}$  cycloalkyl group,  $C_{3-6}$  cycloalkyl)  $C_{1-4}$  alkyl group,  $C_{2-6}$  5 alkenyl group,  $(C_{2-6}$  alkenyl) aryl group, aryl group or heteroaryl group; and  $R_2$  stands for H or a  $C_{1-4}$  alkyl- $C(O)-A-$  or  $C_{1-4}$  alkyl- $NH-C(O)-A$  group, where A stands for

10



p and q are each independently 0 or 1

$R_4$  = H or a  $C_{1-6}$  alkyl group (each  $R_4$  independent of the other one)

15 Y and Z are each independently H or  $(C_{0-4}$  alkyl) $R_5$ , where  $R_5$  is  $NHR_4$ ,  $N(R_4)_2$  ( $R_4$  each independently),  $COOR_4$ ,  $CONHR_4$ ,  $NHCO_2R_4$ ,  $NHSO_2R_4$  or  $NHCOR_4$  and

W is O,  $S(O)_m$ , with  $m$  = 0, 1 or 2, or  $NR_6$

$R_6$  = H,  $C_{1-4}$  alkyl,  $COR_7$ ,  $CO_2R_7$ ,  $CONHR_7$  or  $SO_2R_7$

20  $R_7$  = H,  $C_{1-4}$  alkyl, aryl, heteroaryl,  $(C_{1-4}$  alkyl)aryl or  $(C_{1-4}$  alkyl) heteroaryl

R and S are each independently CH or N.

These compounds can be prepared in a known manner by for instance activating a substituted or non-

25 substituted  $\alpha$ -mercaptopcarboxylic acid and coupling it to the L-leucyl-L-tert.-leucine-N-methylamide dipeptide obtained according to the invention using classical peptide coupling techniques, as for instance described in WO-A-96/11209 and WO-A-97/12902.

The invention will now be elucidated on the basis of examples, without however being restricted thereto.

5 Example I

Preparation of N-formyl-L-leucyl-L-tert.-leucine-N-methylamide from N-formyl-L-leucine and L-tert.-leucine-N-methylamide

Under nitrogen at -18°C

10 isobutylchloroformate (6.5 g, 48 mmol) was dosed to a solution of N-formyl-L-leucine (8.0 g, 50 mmol) in tetrahydrofuran (125 ml). Then N-methyl morpholine (4.8 g, 48 mmol) was added dropwise at such a rate that the temperature remained < -15°C. A precipitate was formed.

15 After stirring had been continued for 15 minutes, a solution of L-tert.-leucine-N-methylamide (6.5 g, 45 mmol) in tetrahydrofuran (50 ml) was added in such a way that the temperature remained < -15°C. Subsequently, stirring was continued for 1 hour at -

20 18°C.

The reaction mixture was heated to 0°C and at this temperature water was added (100 g). Then THF was removed by distillation under vacuum. Isopropyl acetate (75 ml) was added and the pH of the reaction mixture was adjusted to 1.5 using hydrochloric acid. After layer separation, the aqueous phase was twice extracted with 50 and 35 ml isopropyl acetate, respectively. The collected organic phases were then washed with 50 and 25 ml saturated sodium bicarbonate solution and finally with 25 ml water. The organic phase was then evaporated under vacuum.

N-formyl-L-leucyl-L-tert.-leucine-N-

methylamide was obtained in a good yield and with an e.e. (L-leucine fragment) of 99% (HPLC).

Example II

5 Preparation of L-leucyl-L-tert.-leucine-N-methylamide from N-formyl-L-leucyl-L-tert.-leucine-N-methylamide

11.7 g (41 mmol) N-formyl-L-leucyl-L-tert.-leucine-N-methylamide (see Example I) was suspended in 1M HCl (100 ml) and heated to 40°C. After 18 hours' 10 stirring at this temperature (all material went into solution), cooling to room temperature and one extraction with 50 ml isopropyl acetate took place.

15 After layer separation the pH of the aqueous phase was adjusted to 10 using 50% sodium hydroxide solution. Two extractions with isopropyl acetate (75 ml) were performed. The collected organic phases were evaporated under vacuum.

20 The residue was suspended in heptane (75 ml) and heated to 65°C. So much isopropyl acetate was added that everything just dissolved. After crystallization by means of cooling to room temperature and filtration, the material was washed twice with heptane (25 ml) and dried. L-leucyl-L-tert.-leucine-N-methylamide was obtained in a good yield with 25 purity = >98% (HPLC)  
e.e. (L-leucine fragment) = 99% (HPLC)

Example III

30 Preparation of N-formyl-L-leucyl-L-tert.-leucine-N-methylamide from N-formyl-L-leucine and L-tert.-leucine-N-methylamide

Under nitrogen at -15°C

isobutylchloroformate (12.3 g, 90 mmol) was dosed to a suspension of N-formyl-L-leucine (15.9 g, 100 mmol) in isopropyl acetate (85 ml). Subsequently, N-methyl morpholine (9.1 g, 90 mmol) in isopropylacetate (25ml) 5 was added dropwise at such a rate that the temperature remained < -10°C.

After stirring had been continued for 90 minutes, the suspension formed was dosed to a cooled solution of L-tert.-leucine-N-methylamide (13.0 g, 90 10 mmol) in methanol (65 ml) in such a way that the temperature remained < -10°C. Stirring was subsequently continued for 30 minutes at -10°C.

The reaction mixture was heated to room temperature and further stirred at this temperature for 15 2 hours. 100 ml water was added to the reaction mixture and the pH was adjusted to 1.0 using 37% aqueous hydrochloride solution. After layer separation the aqueous phase was rewashed with two times 75 ml isopropyl acetate. The collected organic phases were 20 then washed with 100 and 50 ml saturated sodium carbonate solution, respectively.

The organic phase was then evaporated under vacuum. N-formyl-L-leucyl-L-tert.-leucine-N-methylamide was obtained with an e.e. (L-leucine fragment) of 98% 25 (HPLC).

#### Example IV

Preparation of N-formyl-L-leucyl-L-tert.-leucine-N-methylamide from N-formyl-L-leucine and L-tert.-leucine-N-methylamide

N-formyl-L-leucyl-L-tert.-leucine-N-methylamide was prepared as described in Example III.

but now at temperatures between 0-5°C. N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide was obtained with an e.e. (L-leucine fragment) of 86% (HPLC).

5 Example V

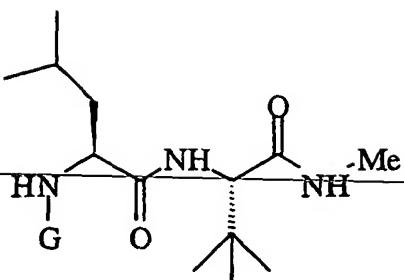
Preparation of L-leucyl-L-*tert.*-leucine-N-methylamide  
from N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide

The material obtained in Example IV was treated as described in Example II. L-leucyl-L-*tert.*-leucine-N-methylamide was obtained with an e.e. (L-leucine fragment) of 95% (HPLC).

C L A I M S

1. Process for the preparation of a dipeptide of formula 1

5



where G represents a protective group  
with N-protected L-leucine being coupled to L-  
10 tert.-leucine-N-methylamide in the presence of an  
activating agent, characterized in that a formyl  
group is used as protective group.

2. Process according to claim 1 in which the L-  
tert.-leucine-N-methylamide has an enantiomeric  
15 excess greater than 98%

3. Process according to claim 1 or 2 in which the N-  
formyl-L-leucine has an enantiomeric excess  
greater than 98%.

4. Process according to any one of claims 1-3 in  
20 which the N-formyl-L-leucyl-L-tert.-leucine-N-  
methylamide obtained is subsequently subjected to  
one or more crystallizations.

5. Process according to any one of claims 1-4 in  
25 which the dipeptide obtained is subsequently  
deformylated.

6. Process according to claim 5 in which the L-  
leucyl-L-tert.-leucine-N-methylamide obtained is

subsequently subjected to one or more crystallizations.

7. Process according to claim 5 or 6 in which the L-leucyl-L-tert.-leucine-N-methylamide is subsequently coupled to a substituted or non-substituted  $\alpha$ -mercaptopcarboxylic acid to form the corresponding N- $\alpha$ -optionally substituted mercaptocarboxyl-L-leucyl-L-tert.-leucine-N-methylamide.

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10 8. N-formyl-L-leucyl-L-tert.-leucine-N-methylamide.

9. N-formyl-L-leucyl-L-tert.-leucine-N-methylamide with an enantiomeric excess of the N-terminal amino acid in the dipeptide of more than 80%.

10. N-formyl-L-leucyl-L-tert.-leucine-N-methylamide

15 with an enantiomeric excess of the N-terminal amino acid in the dipeptide of more than 98%.

11. N-formyl-L-leucyl-L-tert.-leucine-N-methylamide according to claim 9 or 10 with a diastereomeric excess of more than 80%.

20 12. N-formyl-L-leucyl-L-tert.-leucine-N-methylamide according to claim 11 with a diastereomeric excess of more than 98%.

13. Use of N-formyl-L-leucyl-L-tert.-leucine-N-methylamide according to any one of claims 8-12 in the preparation of pharmaceuticals.

25

# INTERNATIONAL SEARCH REPORT

Int'l Application No

PCT/NL 00/00635

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 C07K5/06

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 11209 A (CHIROSCIENCE LTD.) 18 April 1996 (1996-04-18) cited in the application the whole document —	1-13
A	WO 97 12902 A (CHIROSCIENCE LTD.) 10 April 1997 (1997-04-10) cited in the application the whole document —	1-13
A	EP 0 227 301 A (AJINOMOTO) 1 July 1987 (1987-07-01) the whole document —	1-8

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

\* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the international search

26 March 2001

Date of mailing of the international search report

04/04/2001

Name and mailing address of the ISA

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Authorized officer

Masturzo P

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/NL 00/00635

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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ATK

**PATENT COOPERATION TREATY**

**PCT**

REC'D 21 JAN 2002

WIPO PCT

**INTERNATIONAL PRELIMINARY EXAMINATION REPORT**

(PCT Article 36 and Rule 70) 12

Applicant's or agent's file reference 3956WO	<b>FOR FURTHER ACTION</b>	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/NL00/00635	International filing date (day/month/year) 08/09/2000	Priority date (day/month/year) 27/10/1999
International Patent Classification (IPC) or national classification and IPC C07K5		

Applicant DSM N.V. et al.
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1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I  Basis of the report
- II  Priority
- III  Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV  Lack of unity of invention
- V  Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI  Certain documents cited
- VII  Certain defects in the international application
- VIII  Certain observations on the international application

Date of submission of the demand 24/01/2001	Date of completion of this report 18.01.2002
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Name and mailing address of the international preliminary examining authority:	Authorized officer
--	--------------------

European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas	Masturzo, P
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**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/NL00/00635

**I. Basis of the report**

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

**Description, pages:**

1-10 as originally filed

**Claims, No.:**

1-13 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- the description, pages:
- the claims, Nos.:
- the drawings, sheets:

5.  This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/NL00/00635

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes:	Claims	1-13
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-13
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-13
	No:	Claims	

2. Citations and explanations  
see separate sheet

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/NL00/00635

**R Item V**

**Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

Reference is made to the following documents:

D1: WO-A-9712902 (Chiroscience);

D2: WO-A-9611209 (Chiroscience);

D3: EP-A-227301 (Ajinomoto).

The subject matter of the present claims 1-13 is novel, inventive and has industrial applicability under Art. 33(2), (3) and (4) PCT. In fact no process has been described for the formation of the compound of claim 1 using formyl as a protecting group. The only used processes in fact rely on the use of different protecting groups (see D2, intermediate 107 and D1, examples 1-3). The intermediates of claims 8-13 are similarly undisclosed in D1-D2 and as such novel under Art. 33(2) PCT.

If the general objective problem is set as to define an alternative process for the preparation of the compounds of claim 1, it appears to be evident that, among the protecting groups offered by peptide chemistry, formyl is one of the most advantageous ones (D3). However, using it as a protecting group in the preparation of the compounds of claim 1 might lead to racemization, which is an undesired side-effect in the synthesis of peptides. The applicant can demonstrate that the use of formyl in this case does not lead to significant formation of racemate. This was unpredictable from the prior art and therefore claims 1-13 (including also the intermediate compounds) are endowed with inventive step under Art. 33(3) PCT.

## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>3956W0</b>	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. <b>PCT/NL 00/00635</b>	International filing date (day/month/year) <b>08/09/2000</b>	(Earliest) Priority Date (day/month/year) <b>27/10/1999</b>
Applicant <b>DSM N.V. et al.</b>		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 2 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

**1. Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

contained in the international application in written form.

filed together with the international application in computer readable form.

furnished subsequently to this Authority in written form.

furnished subsequently to this Authority in computer readable form.

the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2.  **Certain claims were found unsearchable** (See Box I).

3.  **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

the text is approved as submitted by the applicant.

the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

the text is approved as submitted by the applicant.

the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. —

as suggested by the applicant.

because the applicant failed to suggest a figure.

because this figure better characterizes the invention.

None of the figures.

PCT

**NOTIFICATION OF ELECTION  
(PCT Rule 61.2)**

## From the INTERNATIONAL BUREAU

Date of mailing: 03 May 2001 (03.05.01)	INVENTION ETATS-UNIS D'AMERIQUE in its capacity as elected Office
International application No.: PCT/NL00/00635	Applicant's or agent's file reference: 3956WO
International filing date: 08 September 2000 (08.09.00)	Priority date: 27 October 1999 (27.10.99)
Applicant: BOESTEN, Wilhelmus, Hubertus, Joseph et al	

1. The designated Office is hereby notified of its election made:

in the demand filed with the International preliminary Examining Authority on:

24 January 2001 (24.01.01)

in a notice effecting later election filed with the International Bureau on:

2. The election  was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).